REMARKS

I. Status of the Claims

Claims 54-68 and 73-79 remain under examination in this application. Claims 1-53 and 69-72 have been withdrawn from consideration because they are drawn to the unelected subject matter and species. Applicants reserve the right to later examination of unelected species. Claims 54, 58 and 65 have been amended herewith to further clarify the subject matter being claimed, and new claims 80-83 have been added, support for which can be found, for example, in paragraphs [044] and [052]. No new subject matter is introduced by the claim amendments. Based on the above amendments and the following remarks, reconsideration and an indication of allowability are respectfully requested.

II. Claim Rejections – 35 U.S.C. §112

Claims 54-68 and 73-79 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. More particularly, the Office Action alleged that Claim 54 was vague because the preamble of the claim was directed to a method of monitoring a molecule of interest, and the body of the claim was directed to detecting the emission spectrum of a fluorescent label, therefore, a correlation step was alleged to be missing that relates the detected emission spectrum of the fluorescent label to the molecule of interest.

Applicants traverse the rejection, and respectfully point out that Claim 54 is clearly drawn to a method of monitoring a molecule of interest by detecting an emission spectrum of a fluorescent label of the molecule. However, in an effort to advance prosecution, Applicants have amended Claim 54 for clarity to explicitly recite "b) detecting the emission spectrum of the fluorescent label, and c) correlating the detected fluorescent emission spectrum to the presence of

the attached molecule of interest, thereby monitoring the molecule of interest." rendering the rejection moot. Accordingly, Applicants respectfully request the rejection under 35 U.S.C. §112, 2nd paragraph, be withdrawn.

III. Claim Rejections – 35 U.S.C. §102

Claims 54-58 and 73 were rejected under 35 U.S.C. §102(b) as being anticipated by Leuvering (U.S. Patent No. 4,313,734). The Office Action alleged that Leuvering discloses methods of immunoassay for detection of a desired analyte using nanocluster of encapsulated noble metal particles, which can be composed of gold, silver, or copper, and can be encapsulated with protein, such as albumin or protein A. Leuvering allegedly further discloses that the nanoclusters contain two or more noble metal particles, and detection of the nanoclusters can be done colorimetrically. With respect to claims 57-59 and 62-65, the Office Action alleged that since the nanoclusters of Leuvering are composed of the same materials as the instantly claimed nanoclusters, they will exhibit the same properties as those recited in claims 57-59 and 62-65. Applicants traverse the rejection as follows.

Applicants respectfully point out that Leuvering does not anticipate claims 54-58 and 73 because Leuvering discloses a process of colorimetrically detecting nanoparticles having a particle size of at least 5 nm, and preferably 10 to 100 nm (Leuvering, Col. 2, lines 35-36 and 51-52). The present invention, as defined in the presently amended claims, is directed to the use of a water-soluble fluorescent label comprising an encapsulated noble metal nanocluster, wherein the noble metal nanocluster is about 0.1 nm to 2 nm in diameter without encapsulation, and wherein the fluorescent label produces fluorescence with a characteristic emission spectrum.

Therefore, there is no overlap in the sizes of the Leuvering nanoparticles which are in excess of 5 nm before being coated with the antibodies, and the noble metal nanoclusters provided by the present invention which are between about 0.1 nm to 2 nm without

encapsulation. The present application clearly distinguishes the claimed nanoclusters from the larger nanoparticles in the prior art, which have a diameter of 3 nm to 100 nm, (*see* Specification, paragraph [045]). Moreover, this size difference is critical in the functional ability of the claimed nanoclusters to create a detectable fluorescence, rather than a colorimetric indicator as in the prior art.

Leuvering's larger nanoparticles rely on colorimetric detection, or absorption at a particular wavelength, and not fluorescence. The strong absorptions of metal particles, as described in Leuvering, are due to plasmon absorption by the free electrons of these metals. For gold this absorption appears around 520 nm (giving a red or pink solution), and for silver it is around 400-420 nm (usually giving a pale yellow solution). Larger metal aggregate sizes produce different colors. This effect is due to (white) light containing all the colors, and the specific wavelengths at which the nanoparticles absorb attenuate these from white light, giving an apparent color of whatever remains. For example, if a nanoparticle absorbs in the green wavelength range (536 nm), the solution will appear red because that's primarily what is left. Therefore the discussion of colorimetric detection in the Leuvering prior art is clearly not fluorescence.

Further, only gold and silver particles larger than ~2.2nm in diameter begin to show a plasmon absorption necessary for colorimetric effects. Any strong absorption at these plasmon absorptions without emission means the particles are at least that large. In fact, most such particles are well above 10 nm. Typically, the smallest sizes by transmission electron microscopy (TEM) reported in the prior art for gold or silver nanoparticles are 4 nm, and most are much larger. Plasmon absorption is not seen for anything smaller than ~2 nm. Plasmon coupling (colorimetric changes) would also then not occur using the presently claimed particles.

No fluorescence would be observed from any of the metal particle samples in Leuvering. This is again consistent with metal nanoparticles (but not nanoclusters) having a plasmon absorption, for which there needs to be a continuous density of states for the electrons to move freely at optical excitation frequencies. Such metals do not fluoresce, as the available states

quench all fluorophores near them through energy and/or electron transfer to their continuous set of states.

Furthermore, the Leuvering nanoparticles, which do not fluorescence, but instead rely on colorimetric detection through light absorption, are made using sodium citrate for reduction, which is well-matched to the creation of large (>5 nm) particles. As explained above, this larger size is necessary in the prior art to obtain the intended colorimetric detection. If the particles of Leuvering were smaller than 2 nm, then they would have no colorimetric signal and thus would be useless in their assay. Further, they would be unable to characterize any particles smaller than ~3 nm using their absorption methods. Therefore, Leuvering clearly did not create or enable the claimed smaller noble metal nanoclusters (between 0.1 nm to 2 nm) of the presently claimed invention having substantially different and advantageous properties for observation.

Therefore, Leuvering discloses a process of making and detecting only the larger nanoparticles, but does not teach a process of making or detecting a much smaller noble metal nanocluster of claims 54-58 and 73. Because Leuvering does not teach the nanocluster limitation of claims 54-58 and 73 as defined in the specification, these claims are not anticipated by Leuvering. Accordingly, Applicants respectfully request the rejections to claims 54-58 and 73 under 35 U.S.C. §102(b) as being anticipated by Leuvering be withdrawn.

IV. Claim Rejections – 35 U.S.C. §103

Claims 74 and 76-79 were rejected under 35 U.S.C. §103(a) as being unpatentable over Leuvering in view of Slocki et al. Applicants respectfully traverse the rejection as follows.

As discussed above, Leuvering does not teach or suggest a process for making or using fluorescent labels comprising encapsulated noble metal nanoclusters with much smaller noble metal nanocluster sizes (0.1 nm to 2 nm) than the nanoparticles used in the cited prior art. Such

deficiency is not cured by Slocki et al., which teaches a peptide encapsulated nanocluster surface to assemble a nanocluster-antibody complex through an epitope interface. Neither Leuvering nor Slocki et al., alone or in combination, teaches or suggests the claimed method of monitoring a molecule of interest attached to a fluorescent label comprising an encapsulated noble metal nanocluster.

In fact, Slocik, et al., directly indicates that no fluorescence is observed from their gold or silver clusters. Their plasmon absorption spectra and transmission electron micrographs clearly indicate that only absorptive particles of diameter 4 nm and larger are created, without any fluorescent particles being produced. Despite the Slocik's usage of the term nanocluster, the particles of Slocik are actually nanoparticles, as used in the present application and in the majority of the art, as evidenced by the electron microscopy, size histograms, absorption spectra, and their stated lack of fluorescence (supplementary information Figure S3 caption).

Moreover, Applicants respectfully point out that the claimed method of monitoring a molecule of interest attached to the fluorescent label comprising an encapsulated noble metal nanocluster, wherein the noble metal nanocluster is from 0.1 nm to 2 nm in diameter without encapsulation, provides distinct and surprising advantages over the prior art. In particular, as described in the application, the small size of nanoclusters provides a strong energy absorption and <u>fluorescent</u> emission under weak illumination (30 W/cm^2) (*see* Specification, paragraph [028] and the Examples). As described above, fluorescent emission would not be possible with the larger metal particle sizes (>5 nm) described in Leuvering, nor would the colorimetric absorption detection system of Leuvering be possible in the nanocluster sizes (0.1 to 2 nm) of the present invention.

Furthermore, the smaller nanocluster size provides facile synthesis and conjugation to proteins, and the option to genetically program the labels such that proteins can be directly labeled intracellularly. Using these fluorescent nanoclusters, the instant invention provides the production and characterization of extremely robust, incredibly bright (e.g., up to 20 times brighter than the best organic dyes), photoactivated biological labels that are simultaneously very

small, biocompatible, suitable for specific *in vitro* and *in vivo* labeling and easily observed on the single molecule level with only weak mercury lamp excitation. The brightness of the claimed encapsulated noble metal nanoclusters enables one to easily perform single molecule experiments with standard, inexpensive, lamp-based fluorescence microscopes. Because only a few atoms to a few tens of atoms of a noble metal are necessary to generate extremely bright compounds easily observed on the single molecule level, the proper biocompatible scaffold encapsulating the noble metal nanoclusters makes these very useful and potentially the smallest possible *in vivo* and *in vitro* labels (see Specification, paragraph [037]). Such surprising advantages satisfy a long-felt need for improvements in diagnostics and research tools, as has been readily recognized by those skilled in the art.

Therefore, it would not have been obvious to one of ordinary skill in the art to make the claimed invention. Accordingly, Applicants respectfully request that the rejections to claims 74 and 76-79 under 35 U.S.C. 103(a) as being unpatentable over Leuvering in view of Slocki et al. be withdrawn.

CONCLUSION

Applicants believe that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested. The foregoing is submitted as a full and complete response to the Office Action mailed October 17, 2008.

No fees are believed due at this time. However, please charge any fees that may be due, or credit any overpayment, to Deposit Account 19-5029 (Ref. No.: 17625-0073). In addition, if there are any issues that can be resolved by a telephone conference or an Examiner's amendment, the Examiner is invited and encouraged to call the undersigned attorney at (404) 853-8000.

Respectfully submitted,

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